ISSN (print) 0093-4666 © 2012. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/120.119

Volume 120, pp. 119–125 *April–June 2012*

Dissoconium proteae newly recorded from China

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Abstract — We document the first report of *Dissoconium proteae* from China. This fungus was found on the cuticle of apple fruit collected from orchards in Shaanxi Province. Its morphology is compared with other *Dissoconium* species and a phylogenetic analysis based on ITS sequence is presented.

Key words — sooty blotch and flyspeck, taxonomy, internal transcribed spacer, rDNA

Introduction

Sooty blotch and flyspeck (SBFS) is a worldwide disease complex more than 60 fungi that blemish the cuticle of pome fruits (such as apple) in humid temperate regions and cause cosmetic damage entailing significant economic losses (Batzer et al. 2005). The common name "flyspeck" refers to species in the SBFS complex that appear on fruit surfaces as clusters of black, shiny, round to oval, flattened sclerotium-like bodies with no mycelial mat (Batzer et al. 2005).

The hyphomycete genus *Dissoconium* de Hoog et al. has lightly pigmented conidiophores that produce septate and aseptate conidia in a sympodial manner. The conidia are hyaline, one- or two-celled, and detach forcibly in pairs (Hoog et al. 1983).

Currently the genus includes nine species: *Dissoconium aciculare* de Hoog et al., *D. australiense* Crous & Summerell, *D. commune* Crous & Mansilla, *D. dekkeri* de Hoog & Hijwegen, *D. eucalypti* Crous & Carnegie, *D. mali* G.Y. Sun et al., *D. musae* Arzanlou & Crous, *D. proteae*, and *D. subuliphorum* (Matsush.) R.F. Castañeda (Arzanlou et al. 2008; Crous et al. 1999, 2004, 2007, 2008; Hoog et al. 1983, 1991; Zhang et al. 2007). Batzer et al. (2005) reported five putative *Dissoconium* species within the sooty blotch complex on apple fruit.

Recently, during investigations of SBFS of apple in China, we identified four fungal isolates that represent the first record of *Dissoconium proteae* from China based on ITS sequence analysis and morphological comparison.

Materials & methods

Isolation. In September 2008, apples with flyspeck signs were collected from orchards in Shaanxi Province. The thalli were transferred to a potato dextrose agar slant (potato 200 g, dextrose 20 g, agar 10 g; PDA) directly from the apple surface, and then cultured at 23±1°C in darkness (Sun et al. 2003). Colony description of the species was based on a 1-month-old pure culture. Morphological characteristics are described from specimens obtained from a cover slip inserted into the surface of the media. Voucher specimens and cultures are conserved in the Fungal Herbarium of Northwest A&F University, Yangling, China (HMUABO).

DNA sequencing. Template DNA was extracted from fungal mycelium according to Li et al. (2011); primer pairs used for ITS amplification and sequencing were ITS1-F

Table 1. Sequences used in the phylogenetic analysis

Species	GenBank code	Reference
Dissoconium proteae (mynbxd10)	HM070373	This paper
D. proteae (mynbxd2)	HM070374	This paper
D. proteae (mynbxd1.1)	HM070375	This paper
D. proteae (mynxc1.1)	HM070376	This paper
D. aciculare	AY725519	Crous et al. (2004)
	AY725521	Crous et al. (2004)
D. australiense	EF394854	Crous et al. (2007)
D. eucalypti	EF394855	Crous et al. (2007)
D. mali	EF627451	Zhang et al. (2007)
D. musae	EU514226	Arzanlou et al. (2008)
	EU514225	Arzanlou et al. (2008)
D. proteae	EU707897	Crous et al. (2008)
Dissoconium sp.	AY598874	Batzer et al. (2005)
	AY598875	Batzer et al. (2005)
	AY598877	Batzer et al. (2005)
	FJ425205	Díaz Arias et al. (2010)
Mycosphaerella communis (anam. *D. commune*)	AY725544	Crous et al. (2004)
	DQ302950	Crous et al. (2006)
M. lateralis (anam.: *D. dekkeri*)	EU301078	Burgess et al. (2007)
Ramichloridium apiculatum	EU041794	Arzanlou et al .(2007)
	EU041791	Arzanlou et al .(2007)
R. cerophilum	EU041798	Arzanlou et al. (2007)
R. indicum	EU041799	Arzanlou et al .(2007)
Ramichloridium sp.	FJ425199	Díaz Arias et al. (2010)
Stenella araguata	AF362066	Crous et al. (2001)

(Gardes & Bruns 1993) and ITS4 (White et al. 1990). Amplification was completed with the following cycling parameters: initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 10 min. The PCR products were sequenced by Organism Technology Co., Shanghai, China.

The ITS nucleotide sequences generated in this study were added to sequences downloaded from GenBank (Table 1) that had high similarity according to a BLAST search (National Center for Biotechnology Information's nucleotide blast program). Preliminary alignments were performed using CLUSTAL-X (1.83) (Thompson et al. 1997), imported into BioEdit version 5.0.9.1 (Hall 1999), and manually adjusted. Phylogenetic analysis of aligned DNA sequences was performed with PAUP version 4.0b10 for 32-bit Microsoft Windows (Swofford 2001). Heuristic searches were performed with 1000 random sequence additions. Clade stability was evaluated by 1000 bootstrap replications. Other measures for parsimony, including tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC, respectively), were also calculated. *Stenella araguata* was used as the outgroup taxon.

Results: DNA phylogeny

A multiple alignment of the rDNA-ITS was generated with 21 sequences obtained from GenBank plus the sequences of isolates mynbxd10, mynbxd2, mynbxd1.1 and mynxc1.1. An MP tree with 354 length (CI = 0.7232, RI = 0.8622, RC = 0.6235) was constructed (Fig. 1). Two major clades were resolved in the MP trees. One of the major clade with 100% bootstrap value contained three species in *Dissoconium*, *Mycosphaerella,* and *Ramichloridium*. The other major clade also had a bootstrap value of 100%. Our isolates and an authentic *Dissoconium proteae* isolate identified by Crous et al. (2008) fell within a single clade with 71% bootstrap support.

Taxonomy

Dissoconium proteae Crous, Persoonia 20: 68. 2008. Fig. 2

Mycelium external consisting of branched, septate, smooth, hyaline hyphae, 0.8–2.8 µm wide. Conidiophores 9–20 × 2.2–4.7 µm, solitary, arising from hyphae, subcylindrical, subulate, tapering to a bluntly rounded or truncate apex, straight to gently curved, smooth, hyaline, becoming medium pale brown with age, aseptate; conidiogenous loci terminal and lateral, visible as slightly thickened, darkened scars, 0.5 µm wide. Conidia 6.3–11.5 × 2.2–4 µm, solitary, hyaline to pale olivaceous, smooth, ellipsoid, non-constricted to slightly constricted at median septum, apex obtuse, base obconic-truncate, tapering pronounced at somewhat protruding hilum, unthickened, not darkened, 1 µm wide. Secondary conidia developing adjacent to primary conidia, hyaline to subhyaline, aseptate, ellipsoid, tapering prominently towards a protruding, truncate base, 4.3–8 × 1.6–3.8 µm; anastomosing with primary conidia after

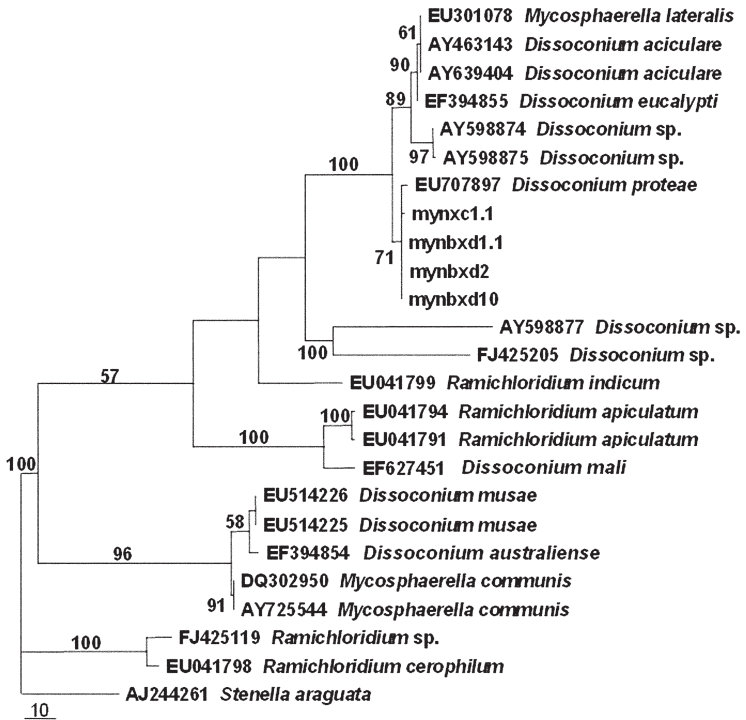

Fig. 1 The majority consensus tree (length = 354, CI = 0.7232, RI = 0.8622, RC = 0.6235) derived from a heuristic search option in PAUP version 4.0b10 for 32-bit Microsoft Windows with 1000 randomizations of sequence input orders and 1000 bootstrap replications using the data set of ITS1, 5.8S and ITS2. Bootstrap values higher than 50% are indicated above or below the tree branches.

active discharge. In some cases the secondary conidia were observed to germinate.

Cultural characteristics: Colonies on PDA spreading, with sparse aerial mycelium and regular smooth margins; surface white or sienna, with patches of white and cinnamon; forming clusters of black sclerotia (remaining infertile) on PDA; reaching 15 mm diam after 1 mo.

Characteristics on the host: On apple peel, the fungus produced dark, shiny, round to oval, slightly protuberant sclerotium-like bodies (Fig. 2A).

specimens examined: China: Shaanxi Province, Xianyang, Bin County, 35°04′N 108°09′E, alt. 1108 m, on *Malus* ×*domestica* (*Rosaceae*), 18 Sep. 2008, Y.N. Mao, HMUABO (the Fungal Herbarium of Northwest A&F University) 8885, 8877, 8866 (with dried culture), culture mynbxd10, mynbxd2, mynbxd1.1. China: Shaanxi Province, Xianyang, Xunyi County, 35°13′N 108°33′E, alt. 1300 m, On *Malus* ×*domestica*, 18 Sep. 2008, Y.N. Mao, HMUABO 8682 (with dried culture), culture mynxc1.1.

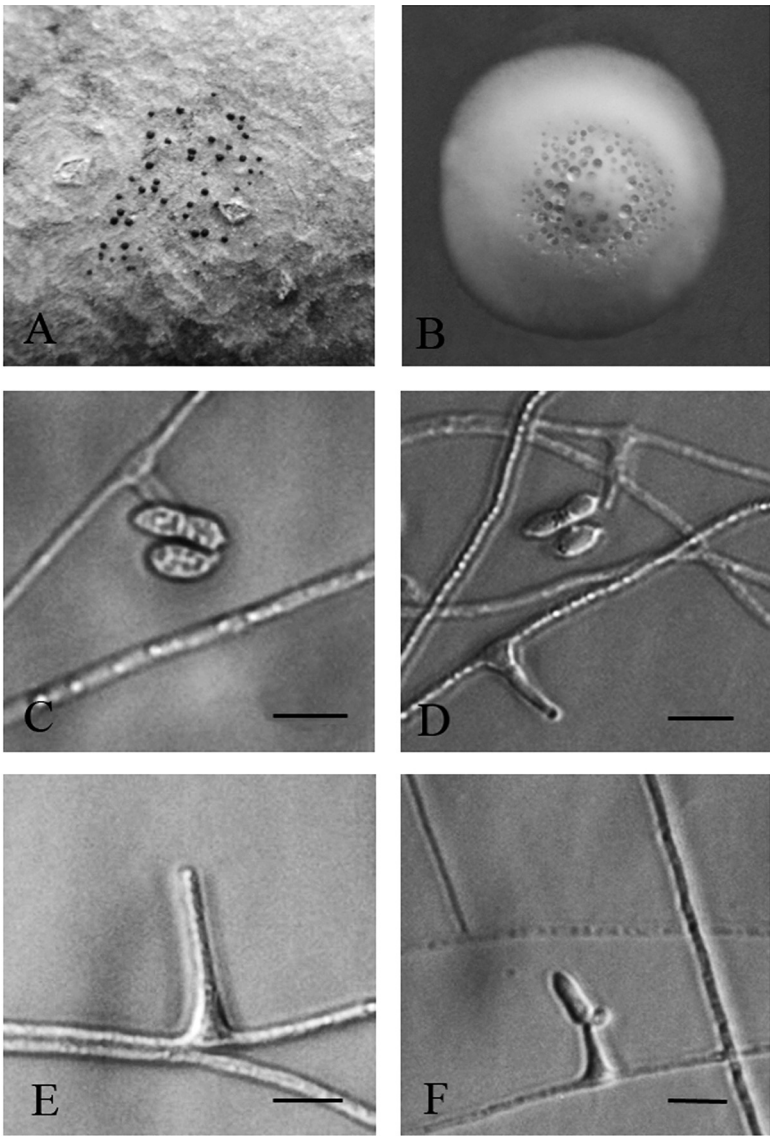

Fig. 2 *Dissoconium proteae* isolate mynbxd10. A. Signs on apple peel. B. Colony on PDA agar after 30 days. C–D. Conidia, conidiogenous cells, and hyphae. E. Conidiogenous cell. F. Conidium and secondary conidium. Bars: B=10 mm, C–F =10 μm.

Discussion

Of the more than 60 fungal species that blemish the surface of apples worldwide (Díaz Arias et al. 2010), many have been described quite recently. For example, Li et al. (2010) and Ma et al. (2010) reported three species of *Zygophiala* (*Z. cryptogama* and *Z. cylindrica*; *Z. qianensis*) associated with flyspeck on apples. Our report provides the first evidence that *Dissoconium proteae* can cause flyspeck on apple fruit. Crous et al. (2008) described *D. proteae* from leaves of *Protea* sp. (*Proteaceae*), causing a leaf spot.

Currently there are nine species in *Dissoconium*. Based on phylogenetic analysis of the ITS region and morphological characters of the anamorph, we identified four isolates as *Dissoconium proteae*. This is the first report of the species from apple, and a new record for China.

Acknowledgments

This work was supported by National Natural Science Foundation of China (31170015, 31171797), the 111 Project from Education Ministry of China (B07049), Top Talent Project of Northwest A&F University and the earmarked fund for Modern Agro-industry Technology Research System (nycytx-08-04-04).The authors wish to thank Dr Eric H.C. McKenzie (Landcare Research, Auckland, New Zealand) and Professor Zhongyi Zhang (College of Plant Protection, Yunnan Agricultural University, Kunming, Yunnan, China) for reviewing the manuscript.

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